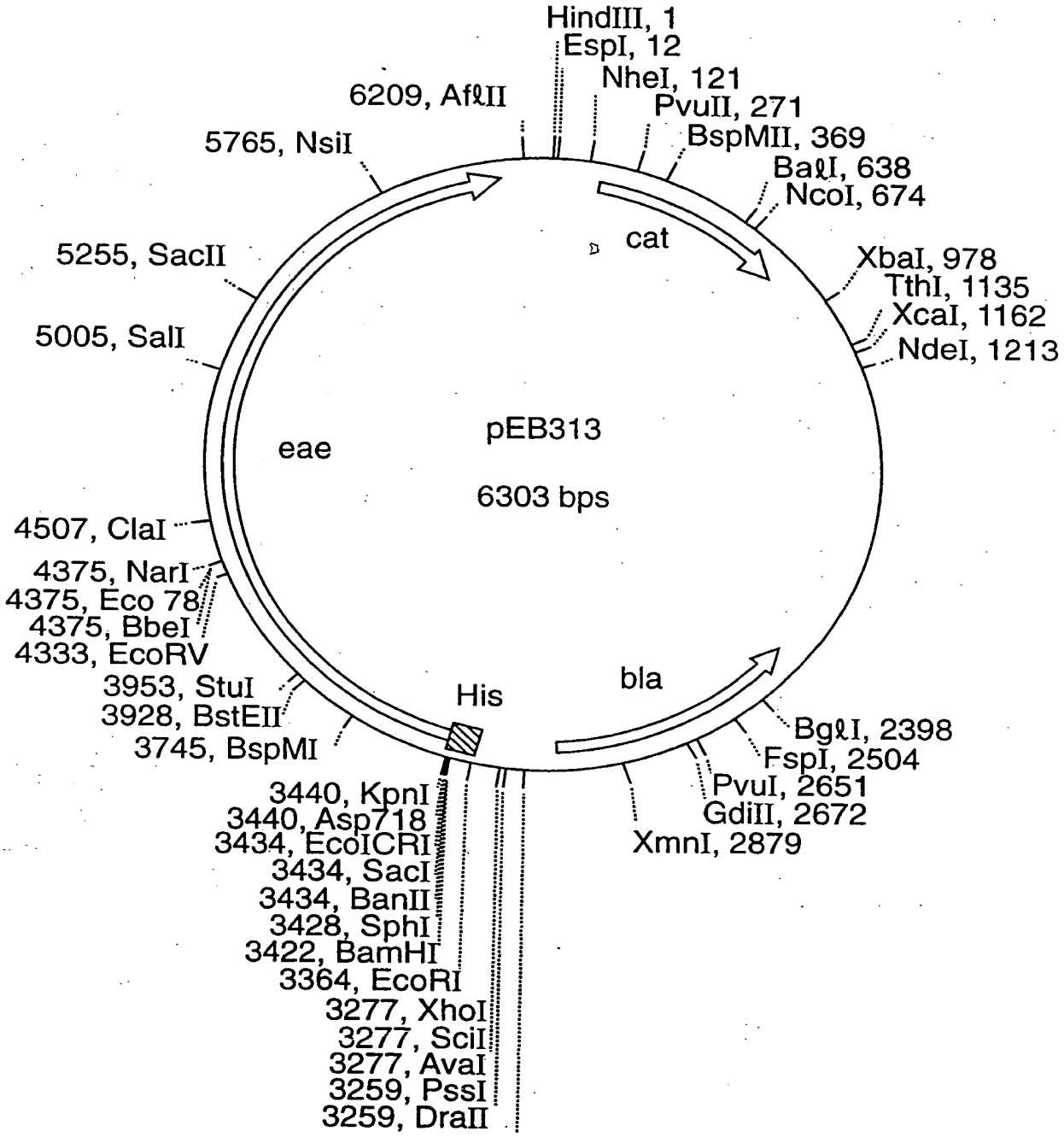

FIG. 1

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1    MITHGCYTRT RHKHKLKKTL IMLSAGLGLF FYVNQNSFAN GENYFKLGSD
51   SKLLTHDSYQ NRLFYTLKTG ETVADLSKSQ DINLSTIWSL NKHLYSSESE
101  MMKAAPGQQI ILPLKKLPFE YSALPLLGSA PLVAAGGVAG HTNKLTKMSP
151  DVTKSNMTDD KALNYAAQQA ASLGSQLQSR SLNGDYAKDT ALGIAGNQAS
201  SQLQAWLQHY GTAEVNLQSG DNFDGSSLDF LLPFYDSEKM LAFGQVGARY
251  IDSRFTANLG AGQRFFLPAN MLGYNVFIDQ DFSGDNTRLG IGGEYWRDYF
301  KSSVNGYFRM RRWHESYHKK DYDERPANGF DIRFNGYLPS YPALGAKLIY
351  EQYYGDNVAL FNSDKLQSNP GAATVGVNYT PIPLVTMGID YRHGTGNEND
401  LLYSMQFRYQ FDKSWSQQIE PQYVNELRTL SGSRYDLVQR NNNIILEYKK
451  QDILSLNIPH DINGTEHSTQ KIQLIVKSKY GLDRIVWDDS ALRSQGGQIQ
501  HSGSQSAQDY QAILPAYVQG GSNIYKVTAR AYDRNGNSSN NVQLTITVLS
551  NGQVVDQVGV TDFTADKTSA KADNADTITY TATVKKNGVA QANVPVSFNI
601  VSGTATLGAN SAKTDANGKA TVTLKSSTPG QVVVSAKTAE MSSALNASAV
651  IFFDQTKASI TEIKADKTTA VANGKDAIKY TVKVMKNGQP VNNQSVTFST
701  NFGMFNGKSQ TQATTGNDGR ATITLTSSSA GKATVSATVS DGAEVKATEV
751  TFFDELKIDN KVDIIGNNVR GELPNIWLQY GQFKLKASGG DGTYSWYSEN
801  TSIATVDASG KVTLNGKGSV VIKATSGDKQ TVSYTIKAPS YMIKVDKQAY
851  YADAMSICKN LLPSTQTVLS DIYDSWGAAN KYSHYSSMNS ITAWIKQTSS
901  EQRSGVSSTY NLITQNPLPG VNVNTPNVYA VCVE (SEQ ID NO:19)
```

FIG. 2

```
1    TCGAGAATGA AATAGAAGTC GTTGTTAAGT CAATGGAAAA CCTGTATTTG GTATTACATA
61   ATCAGGGAAT AACATTAGAA AACGAACATA TGAAAATAGA GGAAATCAGT TCAAGCGACA
121  ATAAACATTA TTACGCCGGA AGATAAAATC CGATCTATTA ATATAATTTA TTTCTCATTC
181  TAACTCATTG TGGTGGAGCC ATAACATGAT TACTCATGGT TGTTATACCC GGACCCGGCA
241  CAAGCATAAG CTAAAAAAAA CATTGATTAT GCTTAGTGCT GGTTTAGGAT TGTTTTTTTA
301  TGTTAATCAG AATTCATTTG CAAATGGTGA AAATTATTTT AAATTGGGTT CGGATTCAAA
361  ACTGTTAACT CATGATAGCT ATCAGAATCG CCTTTTTTAT ACGTTGAAAA CTGGTGAAAC
421  TGTTGCCGAT CTTTCTAAAT CGCAAGATAT TAATTTATCG ACGATTTGGT CGTTGAATAA
481  GCATTTATAC AGTTCTGAAA GCGAAATGAT GAAGGCCGCG CCTGGTCAGC AGATCATTTT
541  GCCACTCAAA AAACTTCCCT TTGAATACAG TGCACTACCA CTTTTAGGTT CGGCACCTCT
601  TGTTGCTGCA GGTGGTGTTG CTGGTCACAC GAATAAACTG ACTAAAATGT CCCCGGACGT
661  GACCAAAAGC AACATGACCG ATGACAAGGC ATTAAATTAT GCGGCACAAC AGGCGGCGAG
721  TCTCGGTAGC CAGCTTCAGT CGCGATCTCT GAACGGCGAT TACGCGAAAG ATACCGCTCT
781  TGGTATCGCT GGTAACCAGG CTTCGTCACA GTTGCAGGCC TGGTTACAAC ATTATGGAAC
841  GGCAGAGGTT AATCTGCAGA GTGGTAATAA CTTTGACGGT AGTTCACTGG ACTTCTTATT
901  ACCGTTCTAT GATTCCGAAA AAATGCTGGC ATTTGGTCAG GTCGGAGCGC GTTACATTGA
961  CTCCCGCTTT ACGGCAAATT TAGGTGCGGG TCAGCGTTTT TTCCTTCCTG CAAACATGTT
1021 GGGCTATAAC GTCTTCATTG ATCAGGATTT TTCTGGTGAT AATACCCGTT TAGGTATTGG
1081 TGGCGAATAC TGGCGAGACT ATTTCAAAAG TAGCGTTAAC GGCTATTTCC GCATGAGCGG
1141 CTGGCATGAG TCATACAATA AGAAAGACTA TGATGAGCGC CCAGCAAATG GCTTCGATAT
1201 CCGTTTTAAT GGCTATCTAC CGTCATATCC GGCATTAGGC GCCAAGCTGA TATATGAGCA
1261 GTATTATGGT GATAATGTTG CTTTGTTTAA TTCTGATAAG CTGCAGTCGA ATCCTGGTGC
1321 GGCGACCGTT GGTGTAAACT ATACTCCGAT TCCTCTGGTG ACGATGGGGA TCGATTACCG
1381 TCATGGTACG GGTAATGAAA ATGATCTCCT TTACTCAATG CAGTTCCGTT ATCAGTTTGA
1441 TAAATCGTGG TCTCAGCAAA TTGAACCACA GTATGTTAAC GAGTTAAGAA CATTATCAGG
1501 CAGCCGTTAC GATCTGGTTC AGCGTAATAA CAATATTATT CTGGAGTACA AGAAGCAGGA
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1681 ACGCAGTCAG GGCGGTCAGA TTCAGCATAG CGGAAGCCAA AGCGCACAAG ACTACCAGGC
1741 TATTTTGCCT GCTTATGTGC AAGGTGGCAG CAATATTTAT AAAGTGACGG CTCGCGCCTA
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1861 TCAAGTTGTC GACCAGGTTG GGGTAACGGA CTTTACGGCG GATAAGACTT CGGCTAAAGC
1921 GGATAACGCC GATACCATTA CTTATACCGC GACGGTGAAA AAGAATGGGG TAGCTCAGGC
1981 TAATGTCCCT GTTTCATTTA ATATTGTTTC AGGAACTGCA ACTCTTGGGG CAAATAGTGC
2041 CAAAACGGAT GCTAACGGTA AGGCAACCGT AACGTTGAAG TCGAGTACGC CAGGACAGGT
2101 CGTCGTGTCT GCTAAAACCG CGGAGATGAC TTCAGCACTT AATGCCAGTG CGGTTATATT
2161 TTTTGATCAA ACCAAGGCCA GCATTACTGA GATTAAGGCT GATAAGACAA CTGCAGTAGC
2221 AAATGGTAAG GATGCTATTA AATATACTGT AAAAGTTATG AAAAACGGTC AGCCAGTTAA
2281 TAATCAATCC GTTACATTCT CAACAAACTT TGGGATGTTC AACGGTAAGT CTCAAACGCA
2341 AGCAACCACG GGAAATGATG GTCGTGCGAC GATAACACTA ACTTCCAGTT CCGCCGGTAA
2401 AGCGACTGTT AGTGCGACAG TCAGTGATGG GGCTGAGGTT AAAGCGACTG AGGTCACTTT
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2581 TGGTACATAT TCATGGTATT CAGAAAATAC CAGTATCGCG ACTGTCGATG CATCAGGGAA
2641 AGTCACTTTG AATGGTAAAG GCAGTGTCGT AATTAAAGCC ACATCTGGTG ATAAGCAAAC
2701 AGTAAGTTAC ACTATAAAAG CACCGTCGTA TATGATAAAA GTGGATAAGC AAGCCTATTA
2761 TGCTGATGCT ATGTCCATTT GCAAAAATTT ATTACCATCC ACACAGACGG TATTGTCAGA
2821 TATTTATGAC TCATGGGGGG CTGCAAATAA ATATAGCCAT TATAGTTCTA TGAACTCAAT
2881 AACTGCTTGG ATTAAACAGA CATCTAGTGA GCAGCGTTCT GGAGTATCAA GCACTTATAA
2941 CCTAATAACA CAAAACCCTC TTCCTGGGGT TAATGTTAAT ACTCCAAATG TCTATGCGGT
3001 TTGTGTAGAA TAATTCCATA ACCACCCCGG CTAAAATATG TATTGTTTTA GTCGGGGCAT
3061 AATTATTTCT TCTTAAGAAA TAACCCTCTT ATAATCAAAT CTACTACTGG TCTTTTTATC
3121 TGCTTAATAG G(SEQ ID NO:20)
```

FIG. 3

```
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61   GCCATAACAT GAGTACTCAT GGTTGTTATA CCCGGACCCG GCACAAGCAT AAGCTAAAAA
121  AAACATTGAT TATGCTTAGT GCTGGTTTAG GATTGTTTTT TTATGTTAAT CAGAATTCAT
181  TTGCAAATGG TGAAAATTAT TTTAAATTGG GTTCGGATTC AAAACTGTTA ACTCATGATA
241  GCTATCAGAA TCGCCTTTTT TATACGTTGA AAACTGGTGA AACTGTTGCC GATCTTTCTA
301  AATCGCAAGA TATTAATTTA TCGACGATTT GGTCGTTGAA TAAGCATTTA TACAGTTCTG
361  AAAGCGAAAT GATGAAGGCC GCGCCTGGTC AGCAGATCAT TTTGCCACTC AAAAAACTTC
421  CCTTTGAATA CAGTGCACTA CCACTTTTAG GTTCGGCACC TCTTGTTGCT GCAGGTGGTG
481  TTGCTGGTCA CACGAATAAA CTGACTAAAA TGTCCCCGGA CGTGACCAAA AGCAACATGA
541  CCGATGACAA GGCATTAAAT TATGCGGCAC AACAGGCGGC GAGTCTCGGT AGCCAGCTTC
601  AGTCGCGATC TCTGAACGGC GATTACGCGA AAGATACCGC TCTTGGTATC GCTGGTAACC
661  AGGCTTCGTC ACAGTTGCAG GCCTGGTTAC AACATTATGG AACGGCAGAG GTTAATCTGC
721  AGAGTGGTGA TAACTTTGAC GGTAGTTCAC TGGACTTCTT ATTACCGTTC TATGATTCCG
781  AAAAAATGCT GGCATTTGGT CAGGTCGGAG CGCGTTACAT TGACTCCCGC TTTACGGCAA
841  ATTTAGGTGC GGGTCAGCGT TTTTTCCTTC CTGCAAACAT GTTGGGCTAT AACGTCTTCA
901  TTGATCAGGA TTTTTCTGGT GATAATACCC GTTTAGGTAT TGGTGGCGAA TACTGGCGAG
961  ACTATTTCAA AAGTAGCGTT AACGGCTATT TCCGCATGAG GCGCTGGCAT GAGTCATACC
1021 ATAAGAAAGA CTATGATGAG CGCCCAGCAA ATGGCTTCGA TATCCGTTTT AATGGCTATC
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1141 TTGCTTTGTT TAATTCTGAT AAGCTGCAGT CGAATCCTGG TGCGGCGACC GTTGGTGTAA
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1261 AAAATGATCT CCTTTACTCA ATGCAGTTCC GTTATCAGTT TGATAAATCG TGGTCTCAGC
1321 AAATTGAACC ACAGTATGTT AACGAGTTAA GAACATTATC AGGCAGCCGT TACGATCTGG
1381 TTCAGCGTAA TAACAATATT ATTCTGGAGT ACAAGAAGCA GGATATTCTT TCTCTGAATA
1441 TTCCGCATGA TATTAATGGT ACTGAACACA GTACGCAGAA GATTCAGTTG ATCGTTAAGA
1501 GCAAATACGG TCTGGATCGT ATCGTCTGGG ATGATAGTGC ATTACGCAGT CAGGGCGGTC
1561 AGATTCAGCA TAGCGGAAGC CAAAGCGCAC AAGACTACCA GGCTATTTTG CCTGCTTATG
1621 TGCAAGGTGG CAGCAATATT TATAAAGTGA CGGCTCGCGC CTATGACCGT AATGGCAATA
1681 GCTCTAACAA TGTACAGCTT ACTATTACCG TTCTGTCGAA TGGTCAAGTT GTCGACCAGG
1741 TTGGGGTAAC GGACTTTACG GCGGATAAGA CTTCGGCTAA AGCGGATAAC GCCGATACCA
1801 TTACTTATAC CGCGACGGTG AAAAAGAATG GGGTAGCTCA GGCTAATGTC CCTGTTTCAT
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1921 GTAAGGCAAC CGTAACGTTG AAGTCGAGTA CGCCAGGACA GGTCGTCGTG TCTGCTAAAA
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2041 CCAGCATTAC TGAGATTAAG GCTGATAAGA CAACTGCAGT AGCAAATGGT AAGGATGCTA
2101 TTAAATATAC TGTAAAAGTT ATGAAAAACG GTCAGCCAGT TAATAATCAA TCCGTTACAT
2161 TCTCAACAAA CTTTGGGATG TTCAACGGTA AGTCTCAAAC GCAAGCAACC ACGGGAAATG
2221 ATGGTCGTGC GACGATAACA CTAACTTCCA GTTCCGCCGG TAAAGCGACT GTTAGTGCGA
2281 CAGTCAGTGA TGGGGCTGAG GTTAAAGCGA CTGAGGTCAC TTTTTTTGAT GAACTGAAAA
2341 TTGACAACAA GGTTGATATT ATTGGTAACA ATGTCAGAGG CGAGTTGCCT AATATTTGGC
2401 TGCAATATGG TCAGTTTAAA CTGAAAGCAA GCGGTGGTGA TGGTACATAT TCATGGTATT
2461 CAGAAAATAC CAGTATCGCG ACTGTCGATG CATCAGGGAA AGTCACTTTG AATGGTAAAG
2521 GCAGTGTCGT AATTAAAGCC ACATCTGGTG ATAAGCAAAC AGTAAGTTAC ACTATAAAAG
2581 CACCGTCGTA TATGATAAAA GTGGATAAGC AAGCCTATTA TGCTGATGCT ATGTCCATTT
2641 GCAAAAATTT ATTACCATCC ACACAGACGG TATTGTCAGA TATTTATGAC TCATGGGGGG
2701 CTGCAAATAA ATATAGCCAT TATAGTTCTA TGAACTCAAT AACTGCTTGG ATTAAACAGA
2761 CATCTAGTGA GCAGCGTTCT GGAGTATCAA GCACTTATAA CCTAATAACA CAAAACCCTC
2821 TTCCTGGGGT TAATGTTAAT ACTCCAAATG TCTATGCGGT TTGTGTAGAA TAATTCCATA
2881 ACCACCCCGG CTAAAATATG TATTGTTTTA GTCGGGGCAT AATTATTTCT TCTTAAGAAA
2941 TAACCTCTTA TAATCAAATC TACTACTGGT CTTTTTATCT GCTTAATAGG TCTCTTTCAA
3001 AGAGACACAT TCACGTTTTC TAGAGTAGGT TGATCCAACC ACGCTGTATA CCAAAGCTGA
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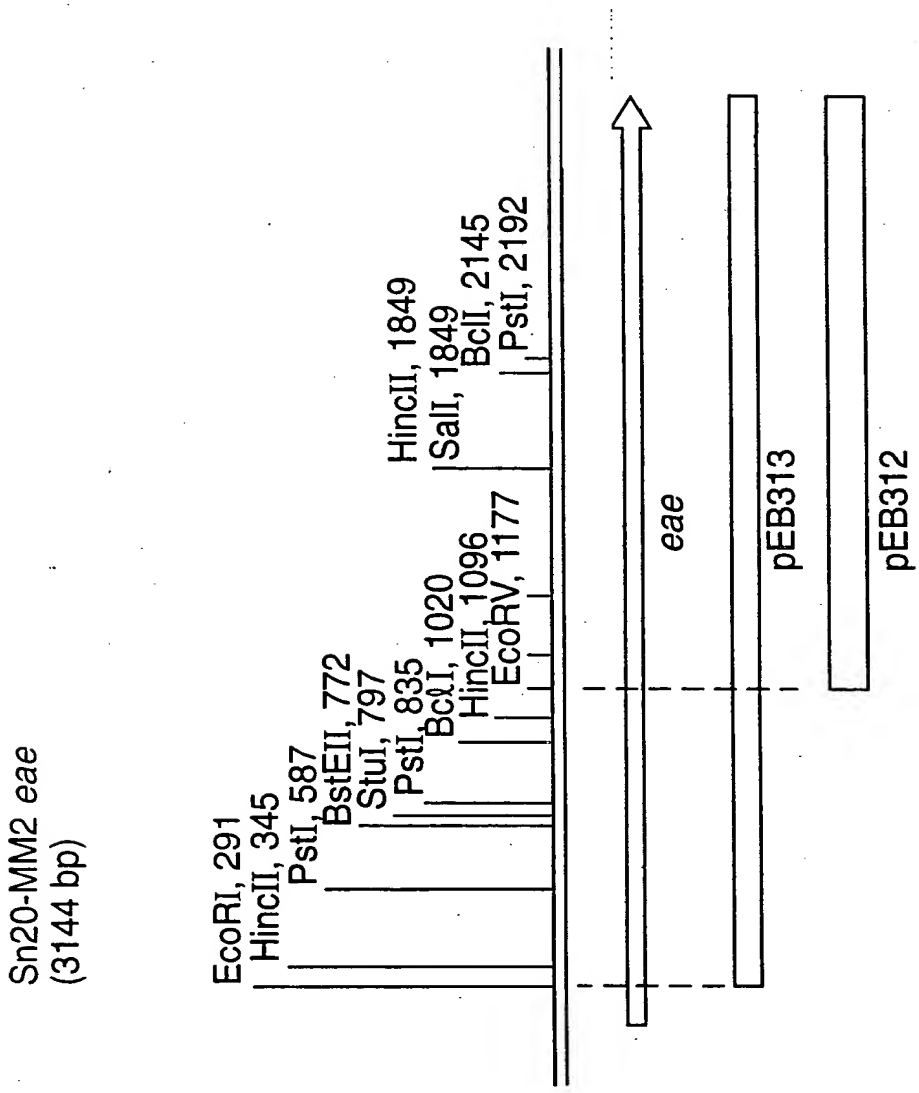
FIG. 4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StuI, 797
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BclI, 1020
HincII, 1096
EcoRV, 1177
HincII, 1849
SalI, 1849
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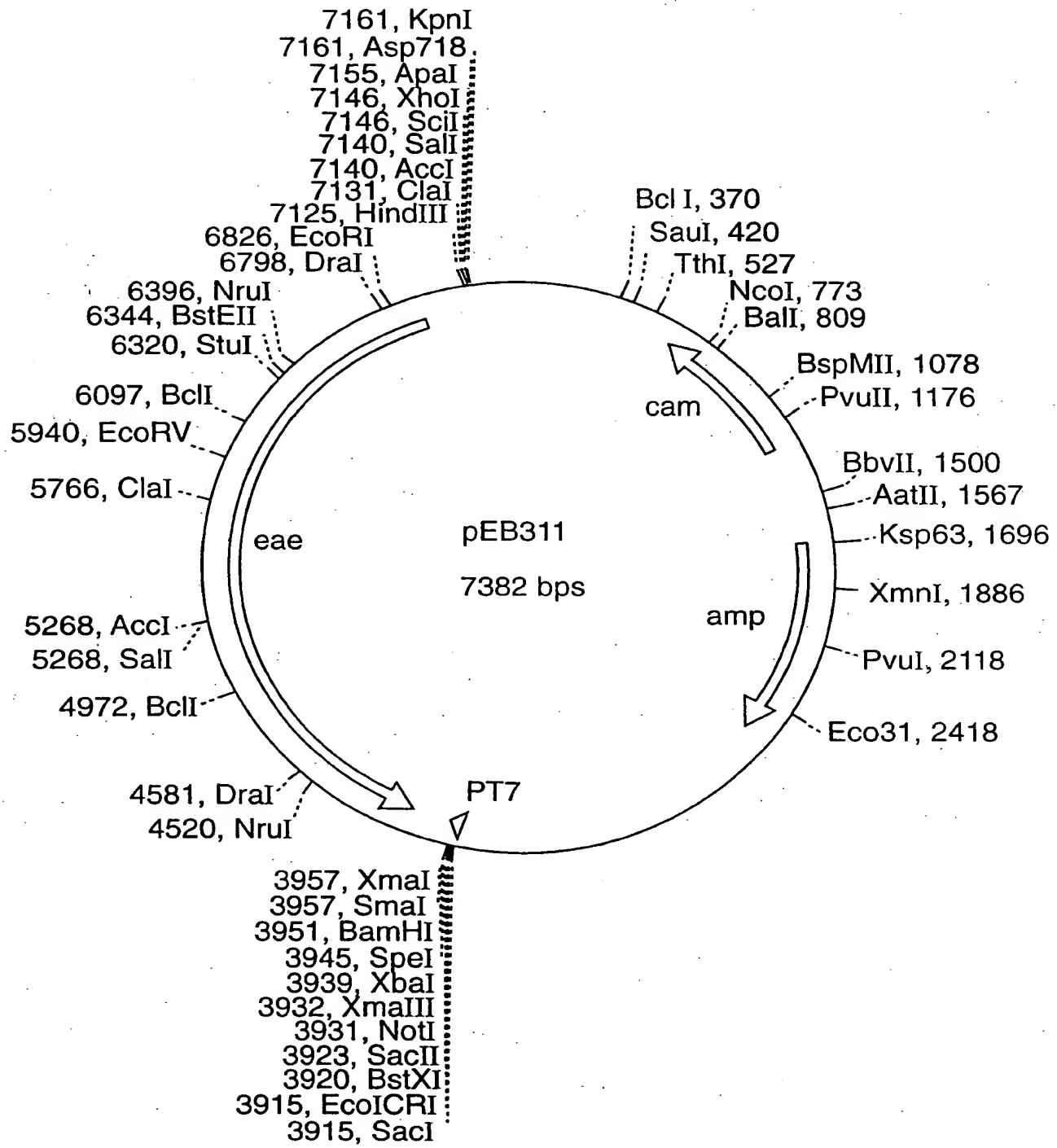
PstI, 2192
eae
pEB313
pEB312

FIG. 5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6826, EcoRI
6798, DraI
6396, NruI
6344, BstEII
6320, StuI
6097, BclI
5940, EcoRV
5766, ClaI
5268, AccI
5268, SalI
4972, BclI
4581, DraI
4520, NruI
3957, XmaI
3957, SmaI
3951, BamHI
3945, SpeI
3939, XbaI
3932, XmaIII
3931, NotI
3923, SacII
3920, BstXI
3915, EcoICRI
3915, SacI
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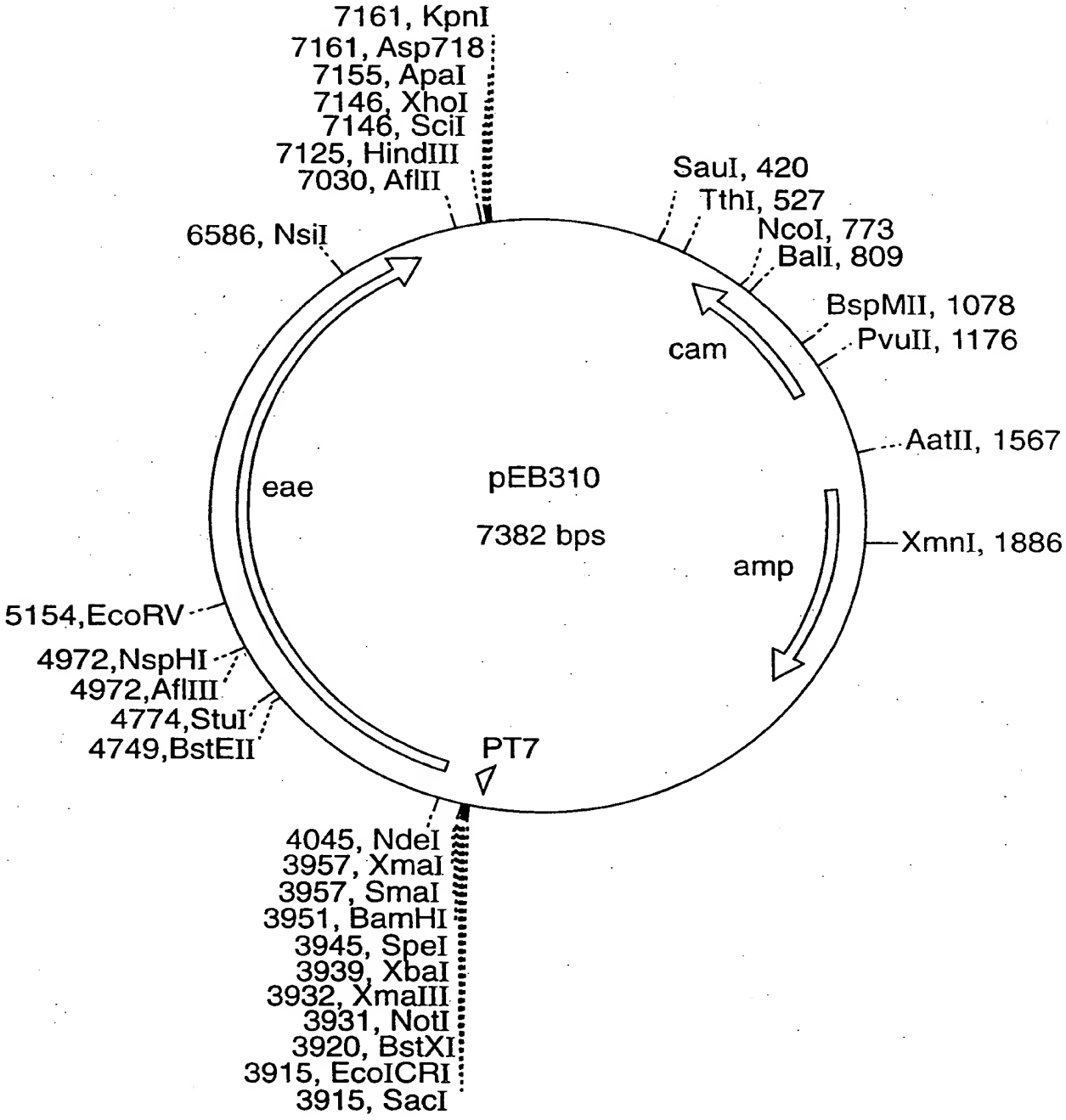
SauI, 420
TthI, 527
NcoI, 773
BalI, 809
BspMII, 1078
PvuII, 1176
BbvII, 1500
AatII, 1567
Ksp63, 1696
XmnI, 1886
PvuI, 2118
Eco31, 2418
cam
amp
eae
pEB311
7382 bps
PT7

FIG. 6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TthI, 527
NcoI, 773
BalI, 809
BspMII, 1078
PvuII, 1176
cam
AatII, 1567
eae
pEB310
7382 bps
amp
XmnI, 1886
5154,EcoRV
4972,NspHI
4972,AflIII
4774,StuI
4749,BstEII
PT7
4045, NdeI
3957, XmaI
3957, SmaI
3951, BamHI
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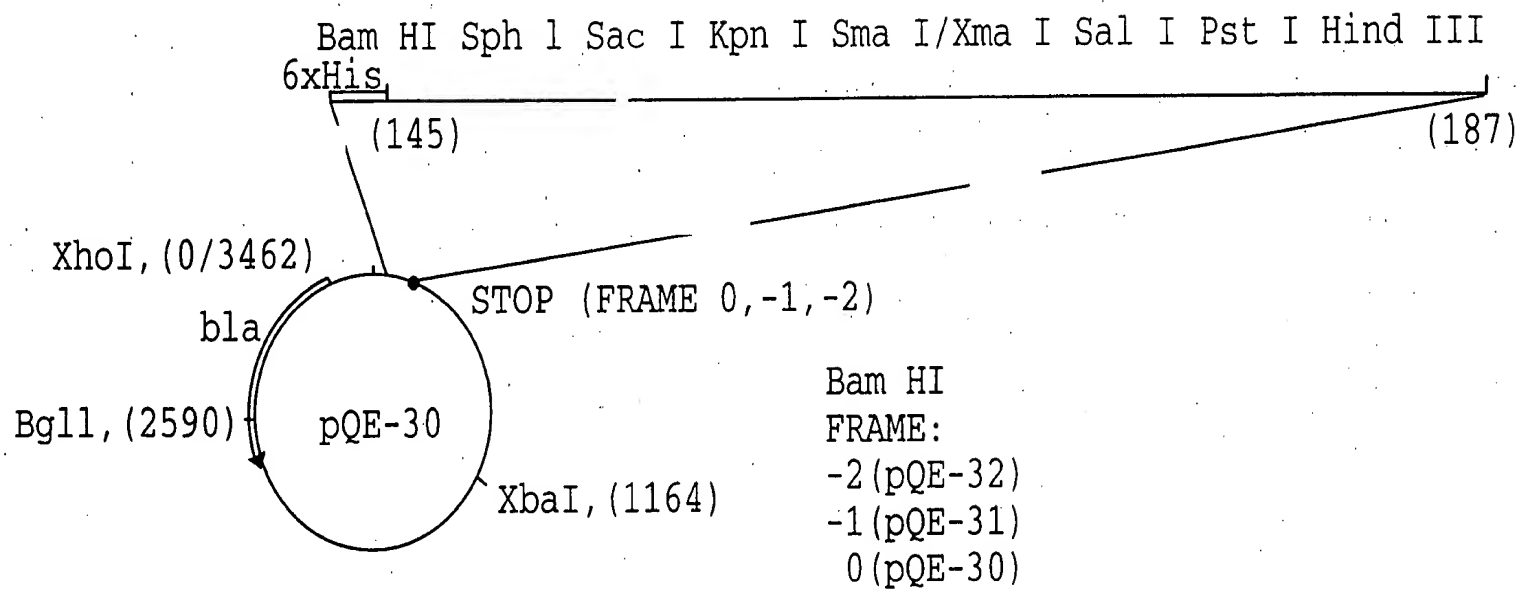
3920, BstXI
3915, EcoICRI
3915, SacI

FIG. 7



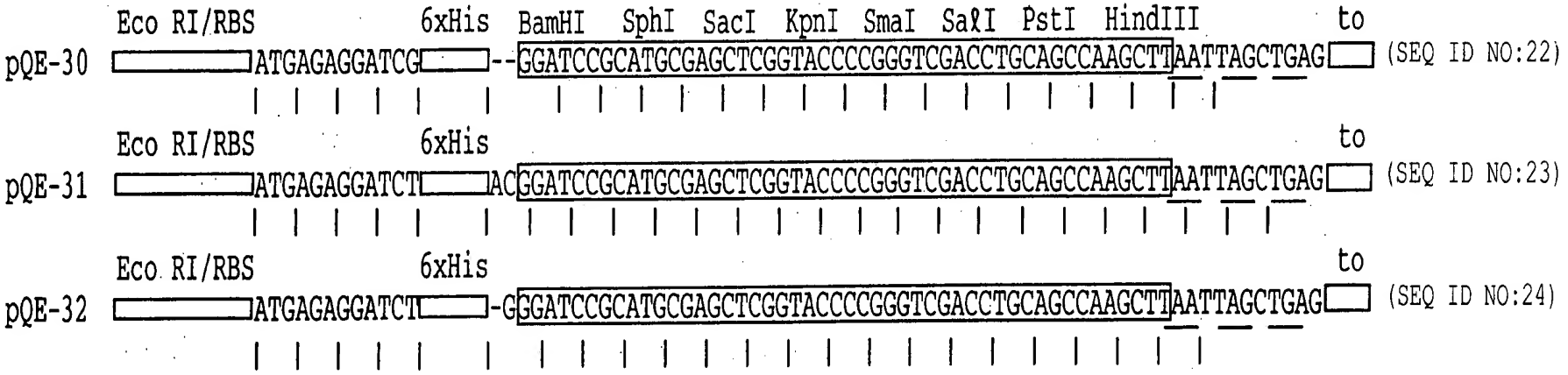


FIG. 8

Replacement Sheet

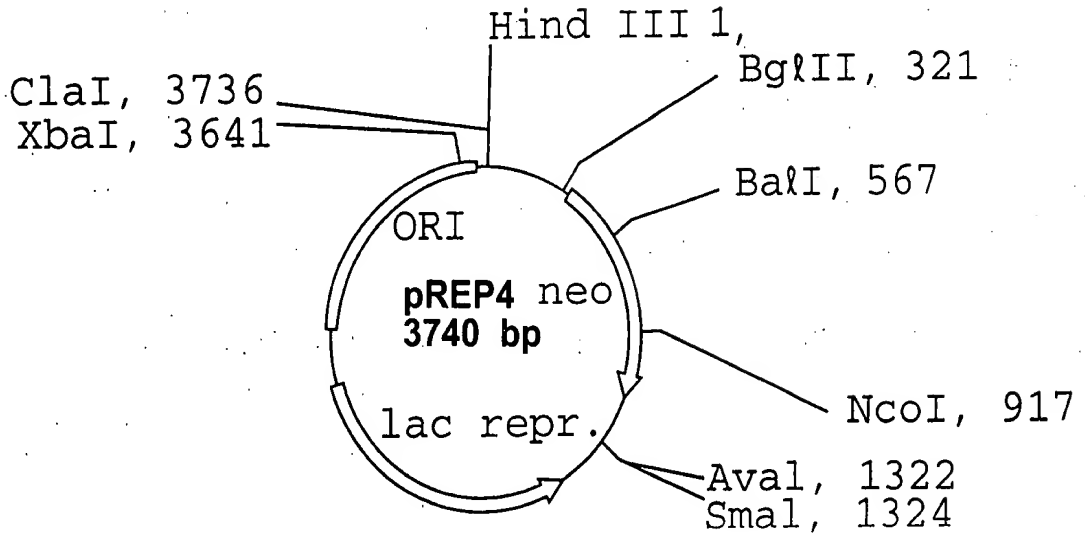


FIG. 9

```
   XhoI                                                operatorI
 1 CTCGAGAAAT CATAAAAAAT TTATTTGCTT TGTGAGCGGA TAACAATTAT
                                                          TATA-Box
                        operator II          EcoRI
51 AATAGATTCA ATTGTGAGCG GATAACAATT TCACACAGAA TTCATTAAAG
             ↑ +1 start mRNA
                    ATG ⟶          6xHis
101 AGGAGAAATT AACTATGAGA GGATCGCATC ACCATCACCA TCACGGATCC
    RBS/SD                                          BamHI

151 GCATGCGAGC TCGGTACCCC GGGTCGACCT GCAGCCAAGC TTAATTAGCT
    SphI   SacI  KpnI  SmaI  SalI  PstI  HindIII
                                              Stop 1 2 3
201 GAGCTTGGAC TCCTGTTGAT AGATCCAGTA ATGACCTCAG AACTCCATCT (SEQ ID NO:25)
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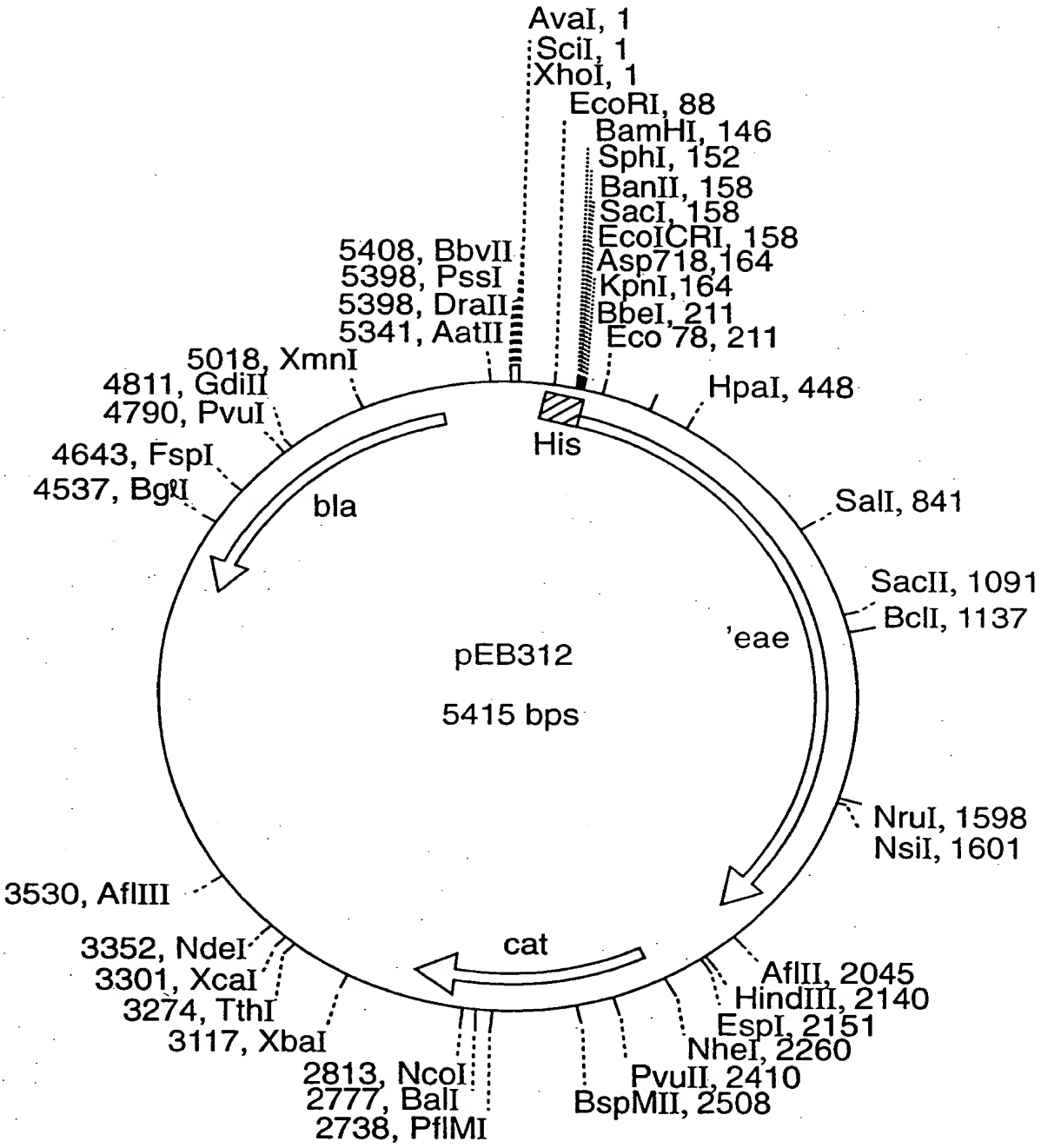
Replacement Sheet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SphI, 152
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SacI, 158
EcoICRI, 158
Asp718, 164
KpnI, 164
BbeI, 211
Eco 78, 211
HpaI, 448
SalI, 841
SacII, 1091
BclI, 1137
'eae
NruI, 1598
NsiI, 1601
AflII, 2045
HindIII, 2140
EspI, 2151
NheI, 2260
PvuII, 2410
BspMII, 2508
2813, NcoI
2777, BalI
2738, PflMI
3352, NdeI
3301, XcaI
3274, TthI
3117, XbaI
3530, AflIII
5018, XmnI
4811, GdiII
4790, PvuI
4643, FspI
4537, BglI
5408, BbvII
5398, PssI
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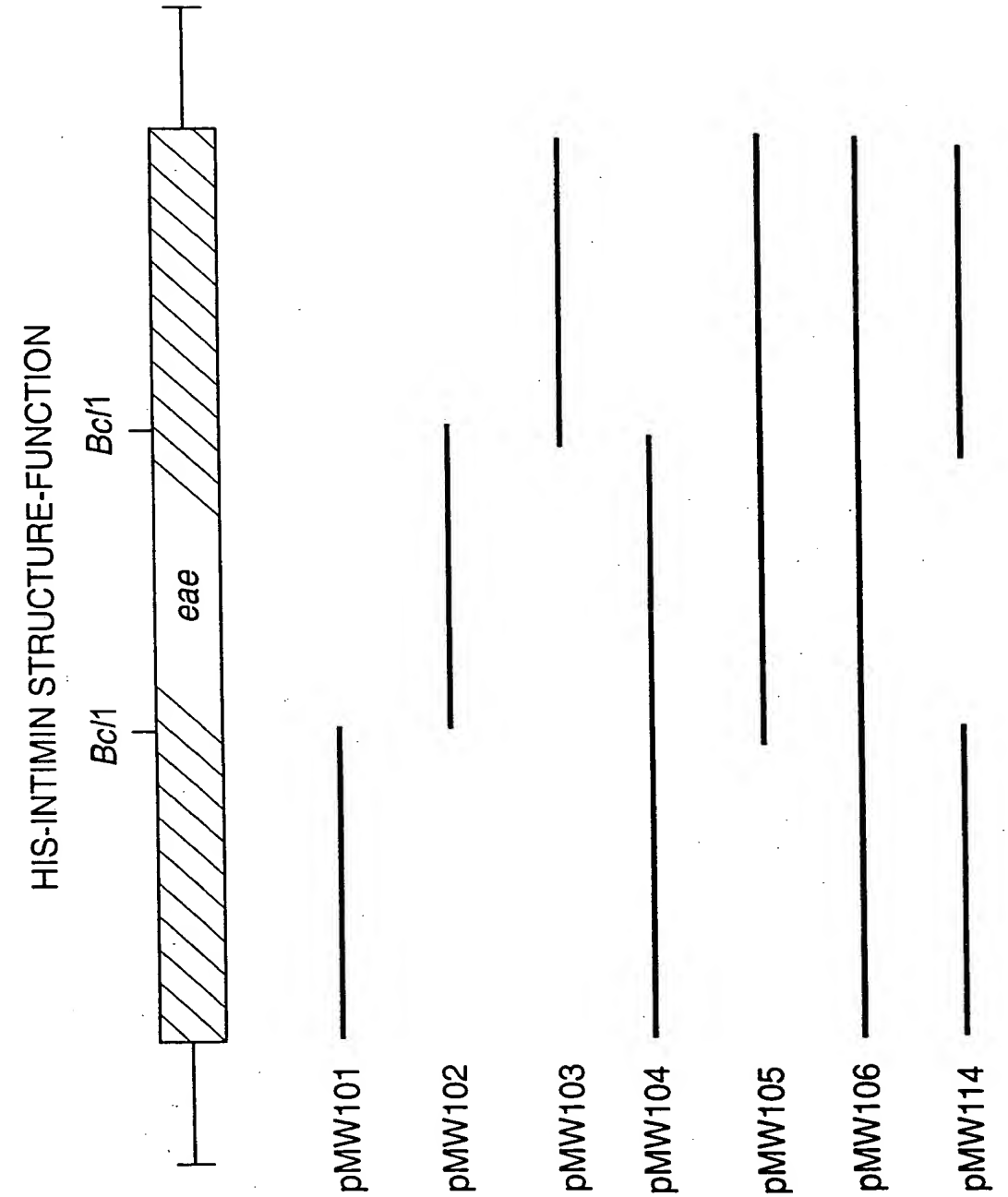
5341, AatII
His
bla
cat
pEB312
5415 bps

FIG. 10


HIS-INTIMIN STRUCTURE-FUNCTION
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eae
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pMW102
pMW103
pMW104
pMW105
pMW106
pMW114

FIG. 11

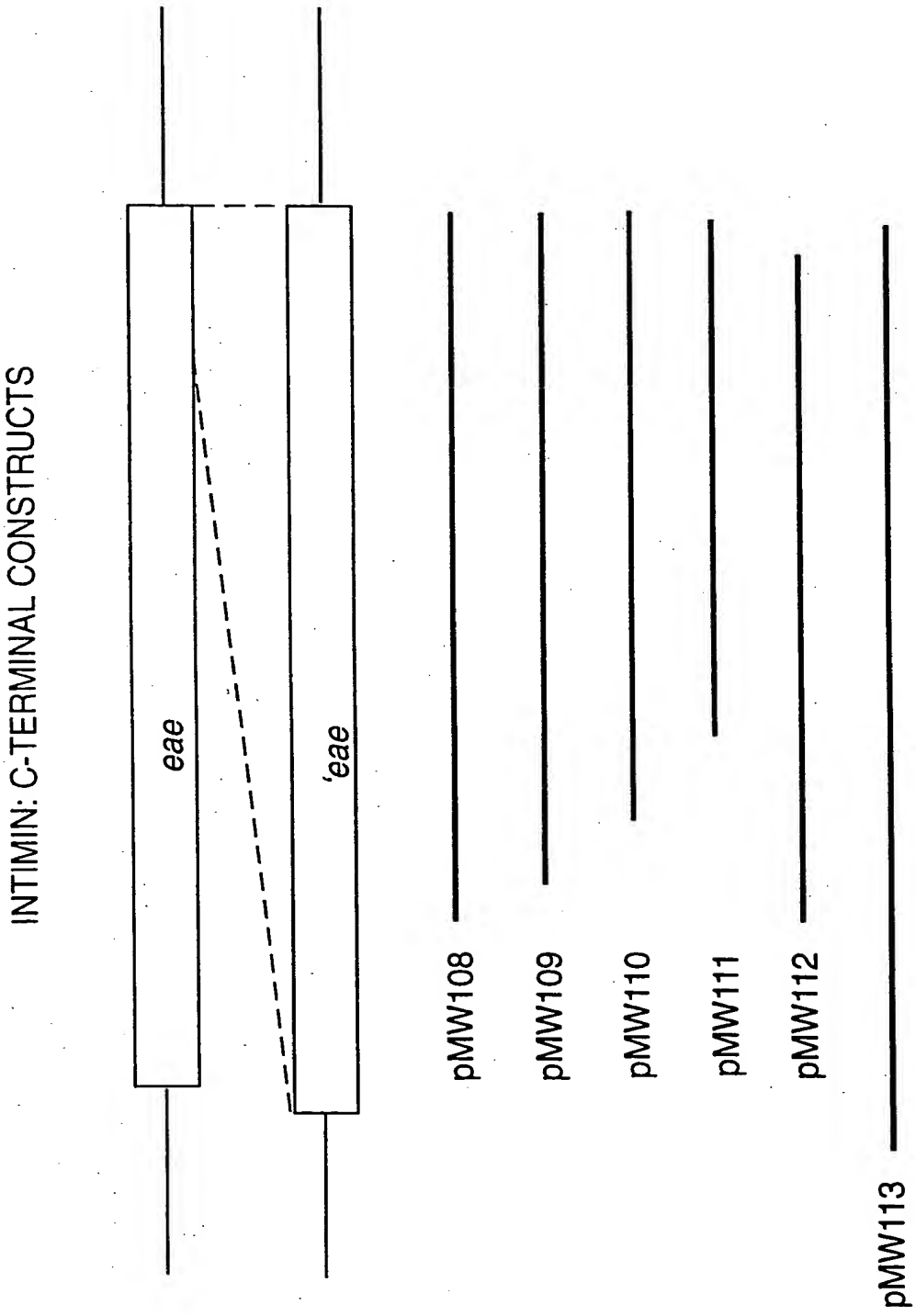
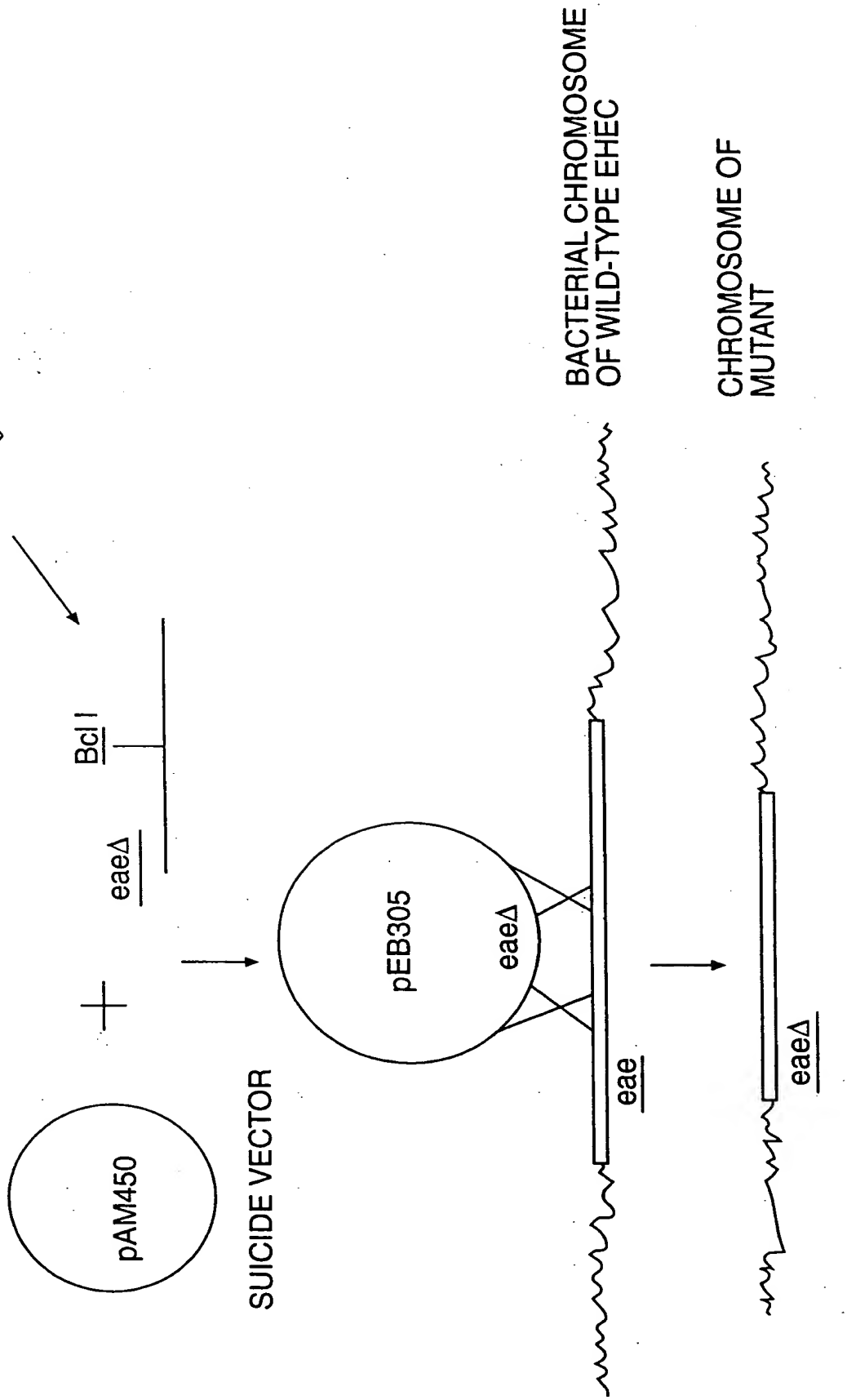

INTIMIN: C-TERMINAL CONSTRUCTS
eae
'eae
pMW108
pMW109
pMW110
pMW111
pMW112
pMW113

FIG. 12

FIG. 13


Bcl I
Bcl I
eae
Bcl I
eaeΔ
pAM450
SUICIDE VECTOR
pEB305
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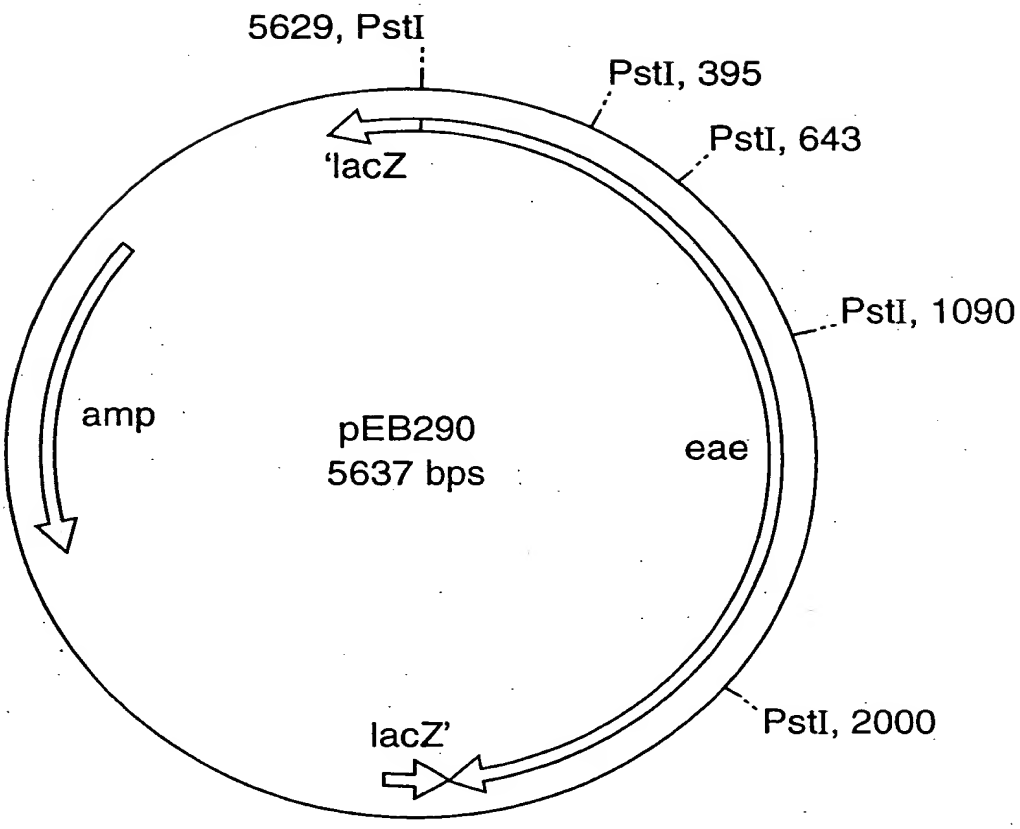
eae
BACTERIAL CHROMOSOME OF WILD-TYPE EHEC
CHROMOSOME OF MUTANT
eaeΔ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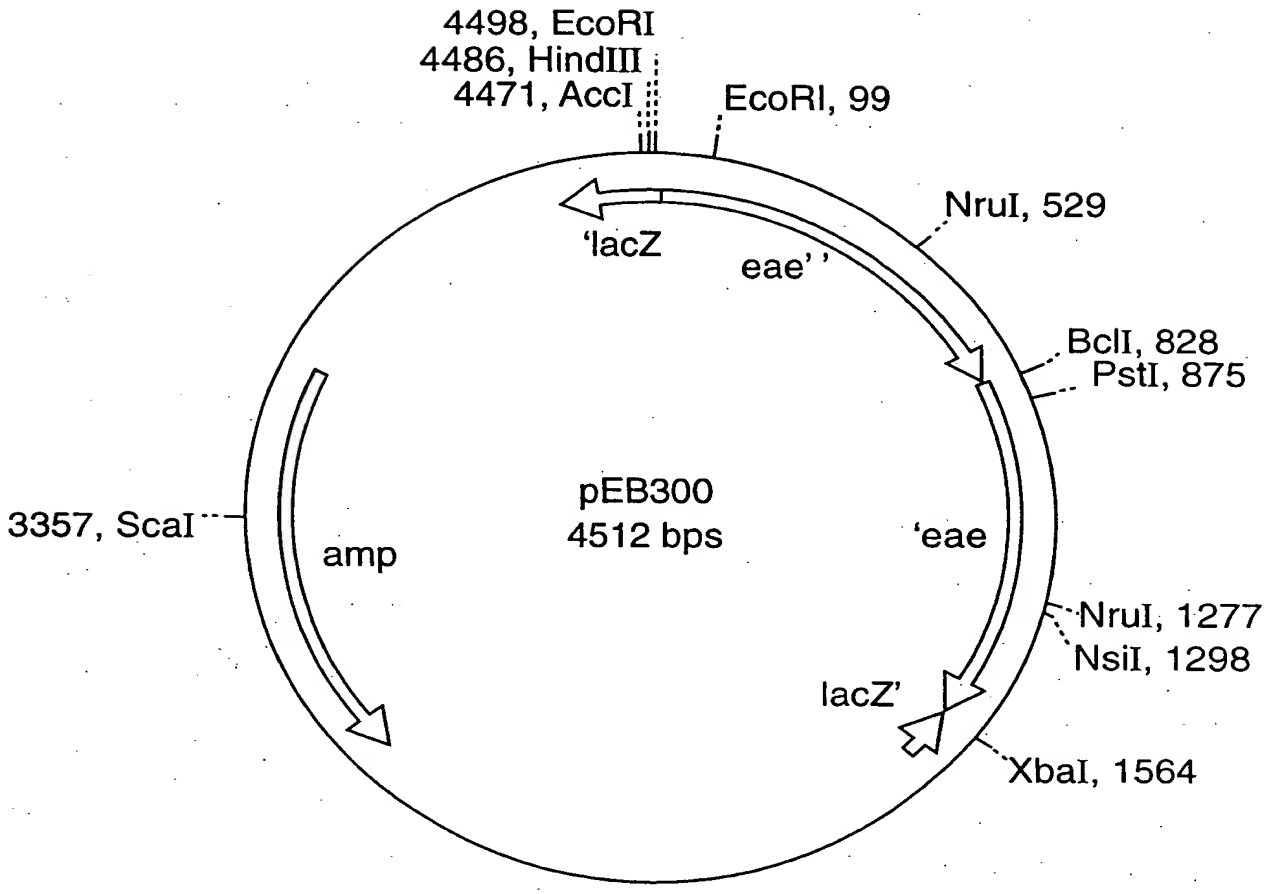
eae
lacZ'
PstI, 2000

FIG. 14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4471, AccI
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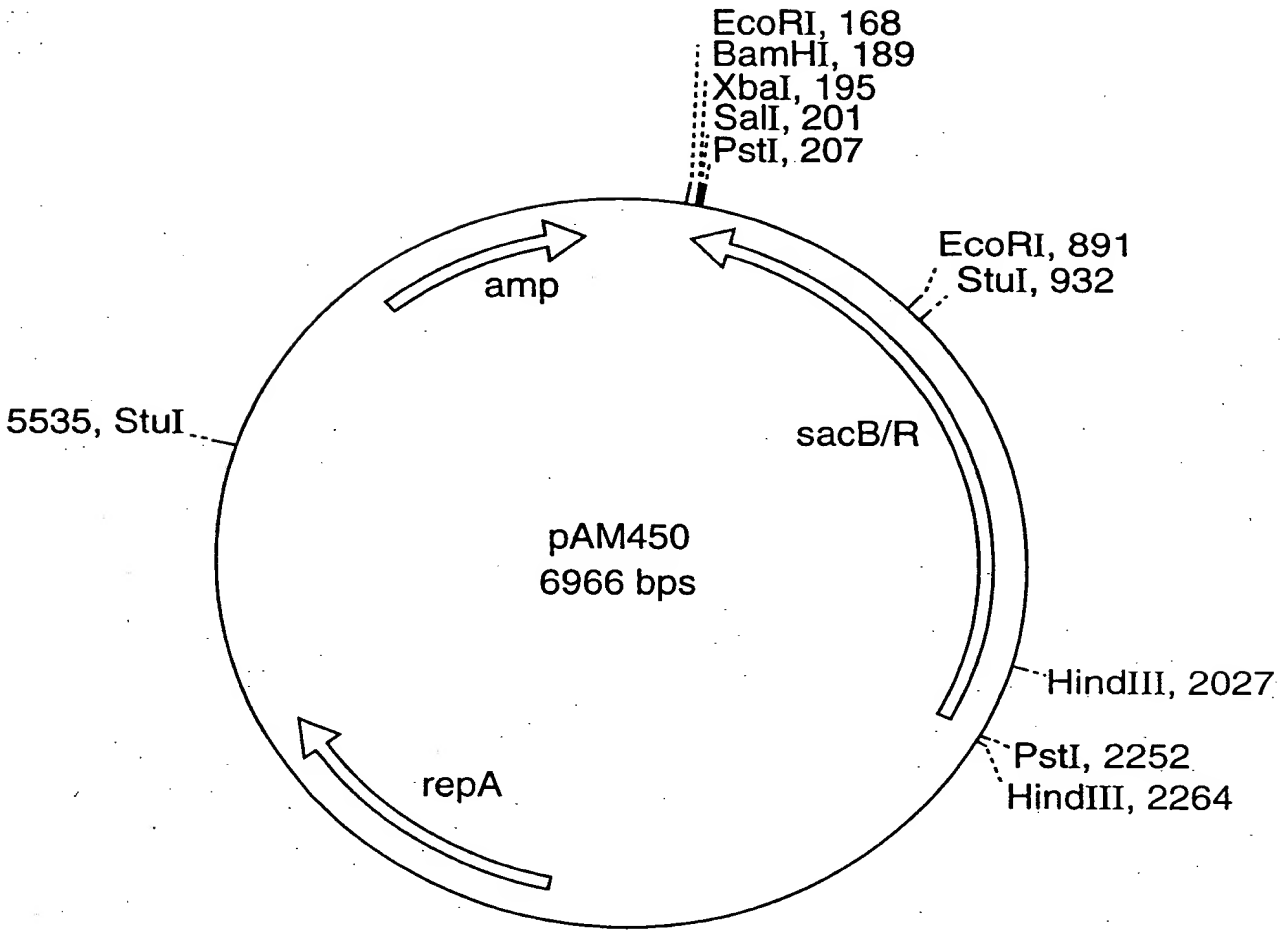
NsiI, 1298
lacZ'
XbaI, 1564

FIG. 15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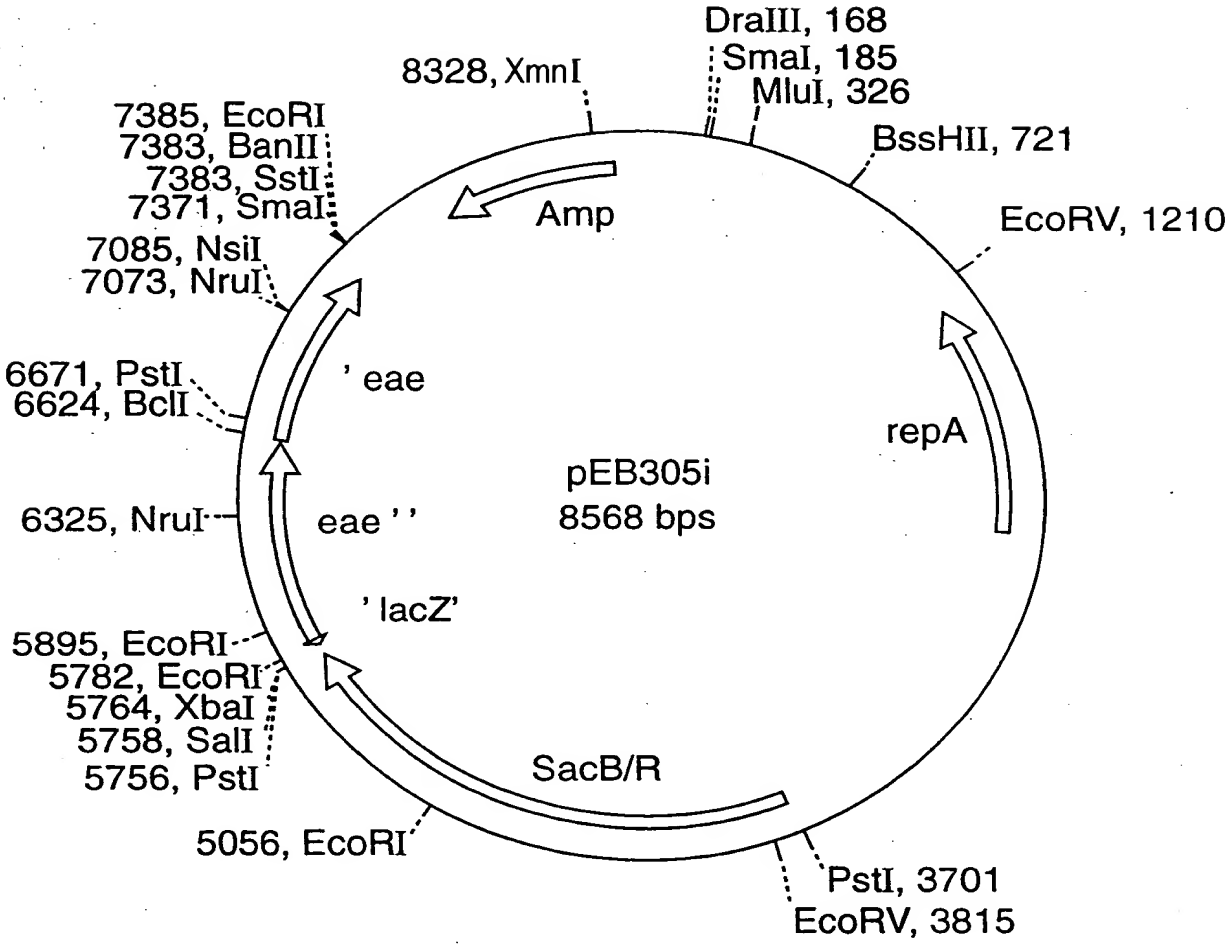
repA

FIG. 16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Amp
EcoRV, 1210
7085, NsiI
7073, NruI
6671, PstI
6624, BclI
' eae
repA
pEB305i
8568 bps
6325, NruI
eae ' '
' lacZ'
5895, EcoRI
5782, EcoRI
5764, XbaI
5758, SalI
5756, PstI
SacB/R
5056, EcoRI
PstI, 3701
EcoRV, 3815

FIG. 17

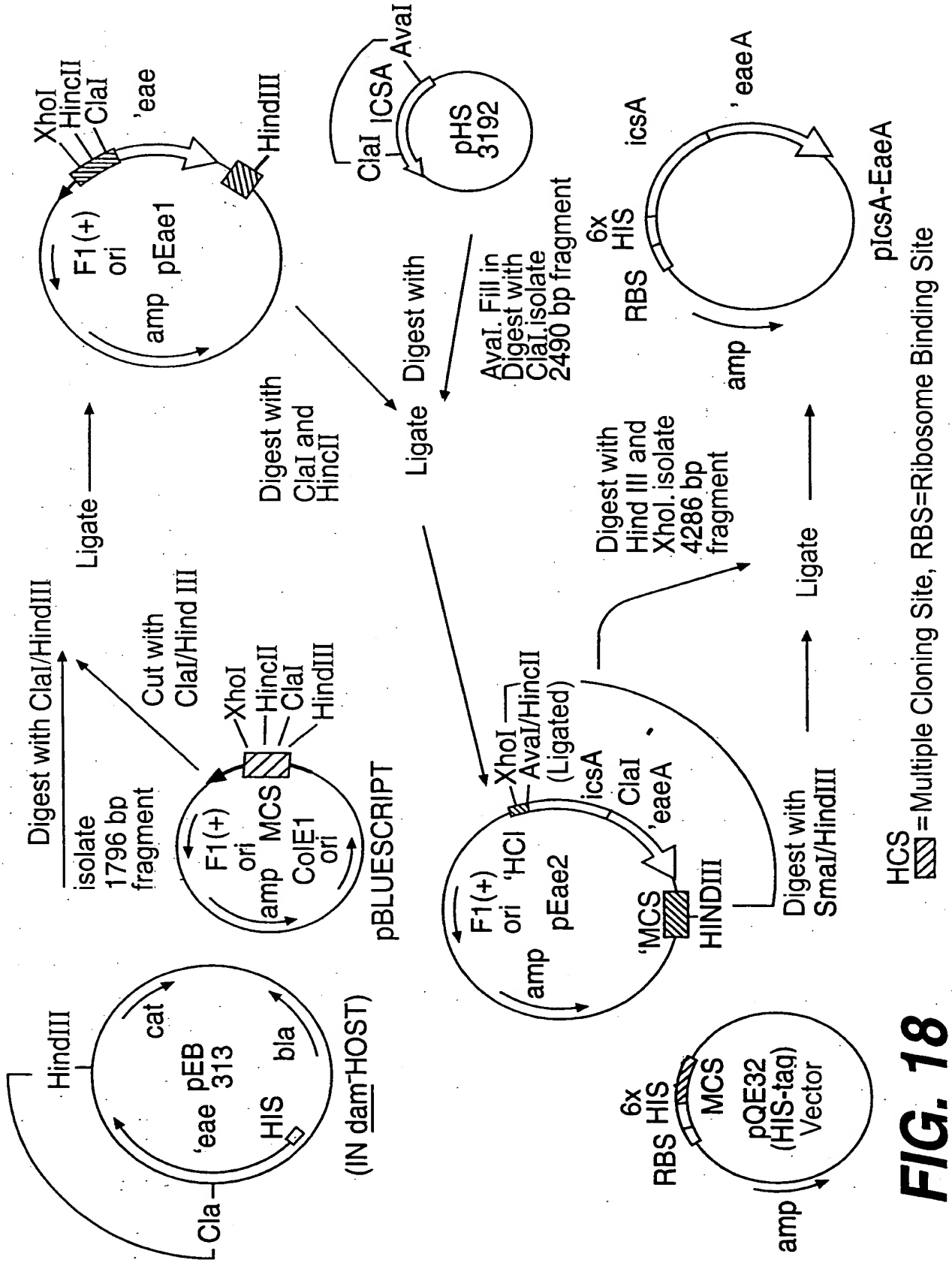
XhoI
HincII
ClaI
'eae
HindIII
F1(+)
ori
amp
pEae1
Digest with ClaI/HindIII
isolate
1796 bp
fragment
Ligate
Cut with
ClaI/Hind III
XhoI
HincII
ClaI
HindIII
F1(+)
ori
amp
MCS
ColE1
ori
pBLUESCRIPT
Digest with
ClaI and
HincII
ClaI
ICSA
AvaI
pHS
3192
Ligate
Digest with
AvaI. Fill in
Digest with
ClaI.isolate
2490 bp fragment
XhoI
AvaI/HincII
(Ligated)
icsA
ClaI
'eaeA
F1(+)
ori
'HCI
amp
pEae2
'MCS
HINDIII
Digest with
Hind III and
XhoI.isolate
4286 bp
fragment
Digest with
SmaI/HindIII
Ligate
6x
HIS
RBS
icsA
'eaeA
amp
pIcsA-EaeA
HindIII
cat
'eae
pEB
313
HIS
bla
Cla
(IN damHOST)
6x
RBS HIS
MCS
pQE32
(HIS-tag)
Vector
amp
HCS
=Multiple Cloning Site, RBS=Ribosome Binding Site

FIG. 18